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Glucagon and prostaglandins are mediators of amino acid-induced rise in renal hemodynamics

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Glucagon and prostaglandins are mediators of amino acid-induced rise in renal hemodynamics. An oral protein load or infusion of amino acids induces a rise in renal hemodynamics in normal subjects, but the mechanisms mediating this phenomenon are unknown. We investigated whether glucagon may mediate the increase in RPF and GFR induced by an arginine infusion and whether prostaglandins are required for this effect. In four different studies, normal subjects underwent 13 inulin and PAH clearances of 30 minutes each. During the fourth and tenth clearance periods arginine HCl, 250 mg/kg, was infused over 30 minutes. At the beginning of the fifth clearance period several subjects ingested indomethacin, 150 mg, ($N = 8$) or ibuprofen, 800 mg, ($N = 6$). Control subjects ($N = 4$) did not receive cyclooxygenase inhibitors. Six subjects underwent a similar protocol except that they were infused with glucagon, 6 ng/kg/min, instead of arginine, for 30 minutes during the fourth and tenth periods. They also ingested indomethacin, 150 mg, in the fifth period. In all four studies, a transient and significant rise in RPF and GFR and fall in RVR occurred during the first arginine or glucagon infusion. These changes in renal hemodynamics were blocked when the arginine or glucagon infusion was repeated after administration of indomethacin or ibuprofen. Urinary excretion of 6-keto-$PGF_{1\alpha}$ did not rise with either arginine infusion in the control subjects or in the individuals who received indomethacin. As predicted, urinary 6-keto-$PGF_{1\alpha}$ fell significantly after ingestion of indomethacin before the second infusion of arginine. Plasma norepinephrine and epinephrine concentrations were unaffected by the arginine infusions or by indomethacin. These findings suggest that glucagon is a mediator of the arginine induced rise in RPF and GFR, and that prostaglandins are necessary for this effect.

It is well documented that the oral intake of protein or the infusion of amino acids induces an acute rise in renal plasma flow (RPF) and glomerular filtration rate (GFR) in normal individuals and most patients with renal disease [1–6]. Two implications have been drawn from these findings that have elicited considerable interest. First, it has been inferred that the increased RPF and GFR after the protein or amino acid load reflect the maximum potential renal function of the kidney [1, 2, 7]. Second, the renal vasodilation and hyperfiltration that occur acutely after a protein load may be caused by the same mechanisms by which a persistently high protein intake induces a chronic increase in RPF, GFR and transcapillary hydraulic pressure [8–12]. Hence, an understanding of the mechanisms by which a high protein diet increases RPF and GFR may lead to a more rational therapy for chronic renal disease.

Since the amino acid-induced increase in renal hemodynamics can be blocked with somatostatin [13–15], this rise is probably mediated by one or more peptide hormones. Growth hormone may increase RPF and GFR chronically [16–18], but it does not seem to have an acute effect on these processes [6, 19]. Glucagon infusions increase RPF and GFR; however, in humans and animals this effect has largely been shown with supraphysiological doses of glucagon [15, 20–22] or with mixtures of glucagon and other hormones [13]. The present study was therefore undertaken to examine whether an infusion of glucagon in quantities that raise plasma hormone levels to postprandial values will acutely increase RPF and GFR. Since certain prostaglandin hormones are vasodilators, this study also investigated whether the effects of an amino acid or glucagon infusion on RPF and GFR are mediated or modulated by eicosanoids.

Methods

Subjects and basic protocol

Twenty normal subjects participated in one or more of the following studies. All were found to be healthy by medical history, physical examination and routine blood chemistry measurements; none of the volunteers had a history of renal disease. Subjects ate self-selected diets prior to participation in the study. Each individual gave written informed consent as approved by the Human Subjects Committee at Harbor-UCLA Medical Center.

Subjects were admitted to the clinical research center at Harbor-UCLA Medical Center at 7:00 a.m. after 12 hours of fasting except for ad libitum water intake. They did not receive any food until the renal function studies were finished. All individuals were maintained at bedrest throughout the study except that they used a commode or stood to urinate.

Body weight and height were measured. A butterfly cannula was inserted into one vein in each forearm. One vein was used for all infusions; the other vein was used for withdrawal of blood samples. Between 7:00 and 8:00 a.m., subjects drank 7.0 ml/kg body weight of deionized water. Between 9:00 a.m. and 3:30 p.m., when urine collections were performed, the volunteers ingested 200 ml of deionized water every 30 minutes to maintain a urine flow of 6 to 10 ml/min. At 8:00 a.m., subjects received an intravenous bolus injection of 50 mg/kg of inulin

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(American Critical Care, McGaw Park, Illinois, USA) and 8 mg/kg of p-aminohippuric acid (PAH, Merck, Sharp and Dohme, West Point, Pennsylvania, USA). Immediately afterwards, an intravenous infusion of inulin, 0.5 mg/kg/min, and PAH, 0.25 mg/kg/min, in normal saline was initiated. This infusion was given at a constant rate of 30 ml/hr by infusion pump, and plasma inulin and PAH levels were maintained at about 25 mg/dl and 2.5 mg/dl, respectively.

After allowing one hour for equilibration, urine was collected by spontaneous voiding every 30 minutes starting at 9:00 a.m. Thus, 13 urine collection periods were performed during a 6.5 hour study. The first three collection periods were used for baseline clearance measurements; the last 10 clearance periods were carried out after the specific infusion protocols, described below, were implemented. In all studies, blood was drawn at the beginning and end of each clearance period in preheparinized tubes for the measurement of hematocrit and plasma inulin and PAH levels. Plasma concentrations of each compound, obtained at the beginning and end of each urine collection period, were averaged for calculating the respective clearances.

Starting with both the fourth and tenth clearance periods (that is, beginning at 10:30 a.m. and 1:30 p.m.), 2 ml of blood were collected on five occasions each, separated by 15 minute intervals, for measurement of plasma glucagon. The blood was collected in glass tubes containing 0.2 ml of aprotinin (Trasylol, Bayer AG, Leverkusen, FRG). In some studies, starting at these same two times (that is, 10:30 a.m. and 1:30 p.m.), 2 ml of blood was collected on four occasions each, separated by 15 minute intervals, for measurement of plasma epinephrine and norepinephrine. In some studies, one ml of heparinized plasma was deproteinized with crystalline sulfosalicylic acid and frozen at −70°C until measured for arginine. An aliquot of the urine, collected in the periods before and during each arginine infusion, was frozen separately at −70°C for the determination of urinary 6-keto-$PGF_{1\alpha}$. All of the above blood samples were drawn on ice and immediately centrifuged. Except as indicated above, plasma and urine samples were stored at −20°C until the various measurements were performed.

At the midpoint of each clearance period, blood pressure was measured with a sphygmomanometer with the subject in a supine position. The mean arterial blood pressure (MABP) was estimated from the systolic (SBP) and diastolic (DBP) pressures as follows:

$$MABP = (SBP - DBP)/3 + DBP$$

Specific protocols

Four different studies were performed using the basic protocol described above.

Study 1 (control study). Three men and one woman with a mean age of 25 years (range, 19 to 34) and body weight of 70.1 kg (range, 54.4 to 88.8) were studied. After the three baseline clearance periods, subjects received an infusion of 10% arginine HCl (R-Gene, Cutter Laboratories, Berkeley, California, USA), 250 mg/kg body weight, over 30 minutes during the fourth clearance period (between 10:30 and 11:00 a.m.). This was followed by five recovery collection periods. During the tenth clearance period (1:30 to 2:00 p.m.), exactly the same infusion was repeated and subjects were then followed for three more collection periods.

Study 2 (arginine-indomethacin-arginine study). Seven men and one woman were studied using the same protocol as described in Study 1. Their mean age was 28 years (range, 19 to 44), and mean body weight was 75.9 kg (range, 54.1 to 89.9). The only difference from Study 1 was that the subjects received a single oral dose of 150 mg of indomethacin (Indocin, Merck, Sharp and Dohme) at 11:30 a.m., 30 minutes after the first arginine infusion and two hours before the second arginine infusion. The same blood and urine collections were performed as in the first study.

Study 3 (arginine-ibuprofen-arginine study). Four men and two women were studied. Their mean age was 26 years (range, 19 to 35), and their body weight averaged 71.0 kg (range, 55.3 to 89.1). This study was identical to Study 2 except that the volunteers received a single oral dose of 800 mg of ibuprofen (Ibuprofen, Boots Laboratories, Inc., Palisades Park, New Jersey, USA) instead of indomethacin. The measurements described in the basic protocol were carried out except that urinary 6-keto-$PGF_{1\alpha}$ and plasma epinephrine, norepinephrine and arginine were not determined, since no major differences from the indomethacin treated group were expected.

Study 4 (glucagon-indomethacin-glucagon). Four men and two women with a mean age of 28 years (range, 21 to 32) and body weight of 70.7 kg (range, 60.6 to 79.7) were studied. Each subject received by infusion pump a constant infusion of glucagon (Eli Lilly Co, Indianapolis, Indiana, USA), 6 ng/kg/min, in 18 ml of normal saline over 30 minutes on two occasions, during the fourth and the tenth clearance periods, respectively. At the end of the fifth period, at 11:30 a.m., subjects ingested 150 mg of indomethacin. Thus, this protocol was exactly the same protocol as in Study 2 except that the arginine infusions were replaced by a constant infusion of a low dose of glucagon. We selected this particular dose of glucagon after having tried higher doses in a pilot study; the currently used dose resulted in an increase in and peak level of plasma glucagon that was very similar to the rise and peak values of glucagon that occurred with the infusions of arginine. All measurements described in Study 3 were performed.

Laboratory analyses

Inulin and PAH concentrations in plasma and urine were determined with the indole method [23] and diazoreaction [24], respectively. Plasma glucagon was measured by radioimmunoassay using the 04A antiserum (Department of Internal Medicine, University of Texas Health Science Center, Dallas, Texas, USA). Plasma epinephrine and norepinephrine were determined as previously described [25]. Plasma arginine levels were measured with an Automated AminoAcid Analyzer Model 121MB (Beckman Instruments, Fullerton, California, USA). Hematocrit was measured by the microhematocrit method.

For determination of urinary 6-keto-$PGF_{1\alpha}$ concentrations, 20 ml of urine were acidified with 1/6 N HCl to a pH of 4.1 to 4.5, spiked with small known amounts of tritiated 6-keto-$PGF_{1\alpha}$ (New England Nuclear, Boston, Massachusetts, USA), and extracted two times with equal volumes of ethylacetate. The organic phase was dried under nitrogen at 37°C, and the dried extract was redissolved in 0.2 ml of the mobile phase (acetonitrile-water-acetic acid-benzine, 30-69.7-0.1-0.2, vol/vol/vol/vol). The redissolved extract was purified with reverse-phase HPLC using a 5 micron C-18 fatty acid analysis column (Waters

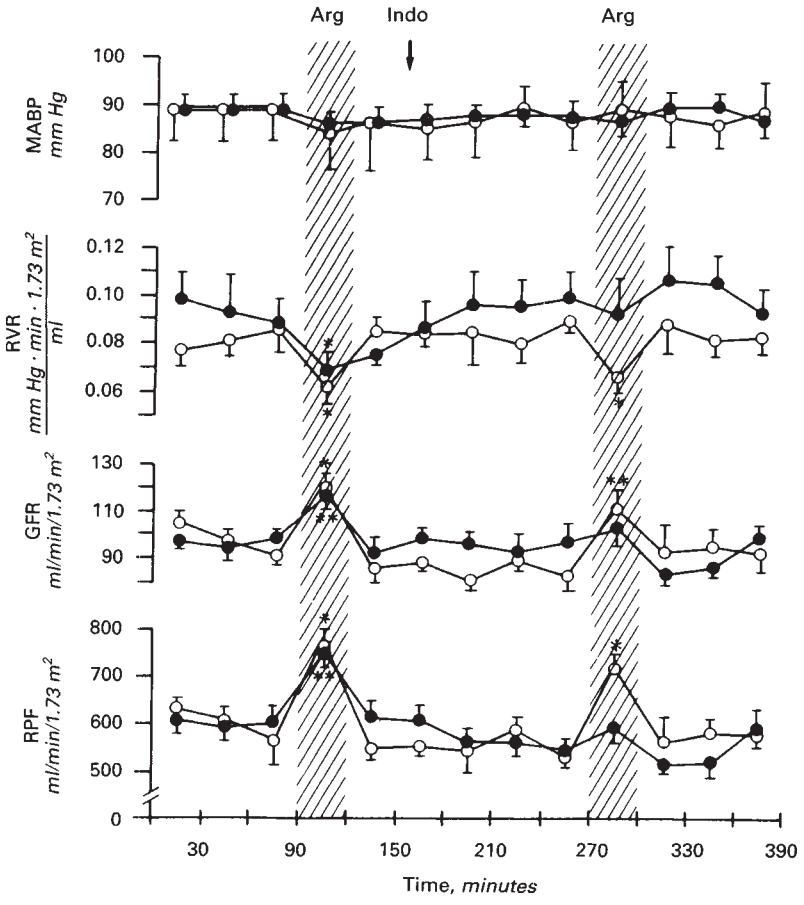

Fig. 1. *Renal plasma flow (RPF), glomerular filtration rate (GFR), total renal vascular resistance (RVR) and mean arterial blood pressure (MABP) in the control subjects (○; Study 1, N = 4) and the subjects, who ingested 150 mg/kg indomethacin between the two arginine HCl infusions (●; Study 2, N = 8).* Arginine HCl was infused during the time indicated by the striped bars. Indomethacin was ingested at the time indicated by the arrow. Significantly different from the mean value of the two baseline clearance periods that immediately preceded the infusion: $^*P < 0.05$.

Assoc., Milford, Massachusetts, USA). The recovery was 31 to 48%. The peak fractions were combined, and a radioimmunoassay was performed using a commercially available ^{125}I-labeled specific antibody (Amersham, Arlington Heights, Illinois, USA). Assay results were corrected for tracer recovery. For further validation, the HPLC peaks of eight samples were also measured by another assay [26]. Results of the two assays were similar ($r = 0.94$). Attempts were also made to measure prostaglandin E_2 in the urine specimens, but for technical reasons this could not be done.

Calculations

GFR was considered to be equal to the inulin clearance. To calculate RPF, PAH clearance values were corrected for the renal extraction of PAH. Since renal vein PAH concentrations were not measured, we assumed that PAH extraction was 85% as previously described [27] and as other investigators have done for normal subjects [28]. Hence, RPF = $C_{PAH}/0.85$. All clearances and flows are expressed per 1.73 $m^2$of body surface area.

Filtration fraction (FF) and total renal vascular resistance (RVR) were calculated from the following equations:

$$FF = GFR/RPF;$$
$$RVR = MABP/RBF \text{ (mm Hg} \cdot \text{min/1.73 m}^2\text{/ml)};$$
$$RBF = RPF/(1 - HCT/100).$$

Statistical analyses for changes in the repetitive measurements were carried out by paired *t*-test. Variance is expressed as standard error of the mean.

Results

In the control study (Study 1, the arginine-arginine infusions), during the first arginine infusion, there was a transient rise in RPF and GFR of +27.6 ± 4.1% ($P < 0.05$)) and +22.9 ± 2.2% ($P < 0.05$), respectively (Fig. 1, Table 1). Both values returned to baseline in the clearance period immediately following the arginine infusion. When the arginine infusion was repeated in period 10, RPF and GFR again increased by +28.5 ± 4.7% ($P < 0.05$) and +27.1 ± 1.0% ($P < 0.01$) (Fig. 1, Table 1). The baseline filtration fraction was 0.16 ± 0.01 and did not change significantly during the study. MABP also did not change significantly during the course of study (Fig. 1). Total renal vascular resistance (RVR) decreased by −25.5 ± 3.7% ($P < 0.05$) and −22.1 ± 3.8% ($P < 0.05$), respectively, during each of the arginine infusions and returned to baseline immediately after each infusion (Fig. 1).

In Study 2 (arginine-indomethacin-arginine), there was a significant increase in RPF (+25.2 ± 2.5%, $P < 0.01$) and GFR (+20.3 ± 2.5%, $P < 0.05$) and a decrease in RVR (−25.7 ± 3.7%, $P < 0.05$) during the first arginine infusion (Fig. 1, Table 1). These changes were transient, and values returned to baseline levels by the first clearance period after cessation of the infusion.

Table 1. RPF and GFR before (baseline), during (0–30 min) and after (30–60 min, 60–90 min) the two infusions of arginine or glucagon

Study	First infusion *min*				Second infusion *min*			
	Baseline	0–30	30–60	60–90	Baseline	0–30	30–60	60–90
Renal plasma flow *ml/min/1.73m²*								
1 ARG-ARG (Control)	601.7 ± 25.3[c]	766.9 ± 33.5[a]	544.8 ± 13.5	552.8 ± 15.9	557.7 ± 18.8	713.3 ± 15.9[a]	566.8 ± 55.8	582.2 ± 24.7
2 ARG-INDO-ARG	603.4 ± 28.8	753.1 ± 30.1[b]	616.2 ± 33.2	608.4 ± 21.0	549.2 ± 22.9	596.8 ± 38.3	513.4 ± 22.1	514.5 ± 36.5
3 ARG-IBU-ARG	576.2 ± 40.5	787.4 ± 57.0[b]	596.8 ± 30.0	585.8 ± 42.2	596.3 ± 36.3	685.0 ± 39.5	506.2 ± 23.0	577.6 ± 32.9
4 GLUC-INDO-GLUC	635.7 ± 31.9	774.9 ± 33.3[a]	657.1 ± 53.1	585.7 ± 32.5	605.0 ± 21.7	657.9 ± 43.4	546.9 ± 26.3	585.9 ± 29.7
Glomerular filtration rate *ml/min/1.73m²*								
1 ARG-ARG (Control)	97.1 ± 3.7	119.4 ± 5.1[a]	84.2 ± 5.2	87.4 ± 2.2	84.8 ± 3.3	111.9 ± 4.3[b]	91.9 ± 8.7	95.0 ± 5.4
2 ARG-INDO-ARG	96.7 ± 2.9	116.3 ± 4.5[b]	92.8 ± 4.4	97.5 ± 3.5	95.8 ± 3.6	102.8 ± 7.5	83.2 ± 5.5	86.6 ± 3.9
3 ARG-IBU-ARG	100.0 ± 3.2	118.7 ± 4.2[a]	97.7 ± 3.7	96.0 ± 3.9	95.5 ± 3.1	97.0 ± 2.0	84.4 ± 2.1	87.5 ± 2.4
4 GLUC-INDO-GLUC	107.6 ± 4.8	125.4 ± 3.5[a]	115.7 ± 5.9	103.3 ± 5.3	107.4 ± 5.7	111.7 ± 2.9	107.8 ± 9.6	103.1 ± 5.1

Different from the respective baseline measurements: [a] $P < 0.05$, [b] $P < 0.01$.
[c] Mean ± SEM.

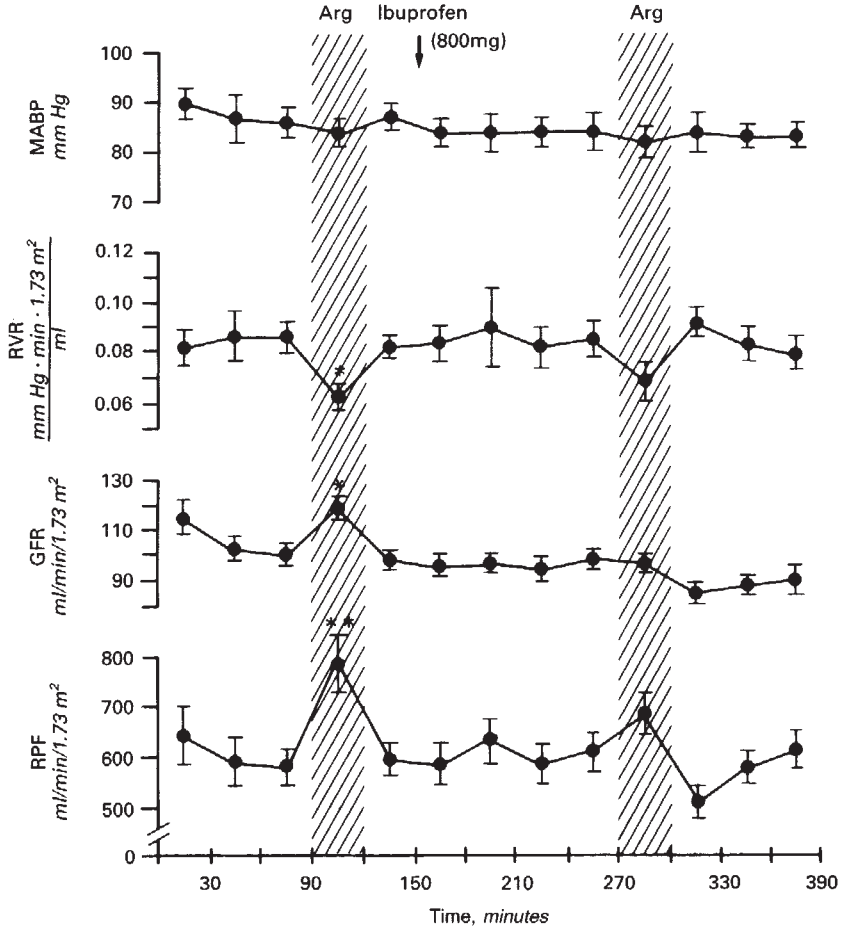

Fig. 2. *Renal hemodynamics and mean arterial blood pressure in the Study 3 subjects who ingested ibuprofen (N = 6).* Symbols are defined in the legend to Figure 1. Significantly different from the mean value of the two baseline clearance periods that immediately preceded the infusion: $*P < 0.05$, $**P < 0.01$.

These results stand in contrast to the changes in renal hemodynamics during the second arginine infusion, after the indomethacin was ingested. There was no change in RPF (+8.4 ± 4.1%, *P*:NS), GFR (+5.4 ± 4.9%, *P*:NS), or RVR (−8.3 ± 4.2%, *P*:NS). However, during the first clearance period after the second arginine infusion was terminated, GFR tended to decrease below baseline levels (*P*:NS); during the next period it had returned to baseline. This phenomenon was not observed for RPF (Fig. 1, Table 1). Filtration fraction did not change significantly during this infusion study.

The baseline plasma arginine levels, measured in Studies 1 and 2, before the first arginine infusion was given, were 85 ± 4 and 70 ± 4 μmol/liter. During the arginine infusion, plasma concentrations rose to 4349 ± 303 and 4146 ± 225 μmol/liter, respectively. Plasma arginine did not return to preinfusion values during the second baseline. Immediately before the second arginine infusion; the plasma concentrations were 426 ± 49 and 377 ± 39 μmol/liter, respectively, in Studies 1 and 2. The second arginine infusion induced a rise in plasma arginine to 4323 ± 432 and 4617 ± 77 μmol/liter, respectively, in the two studies.

Study 3 examined whether another cyclooxygenase inhibitor

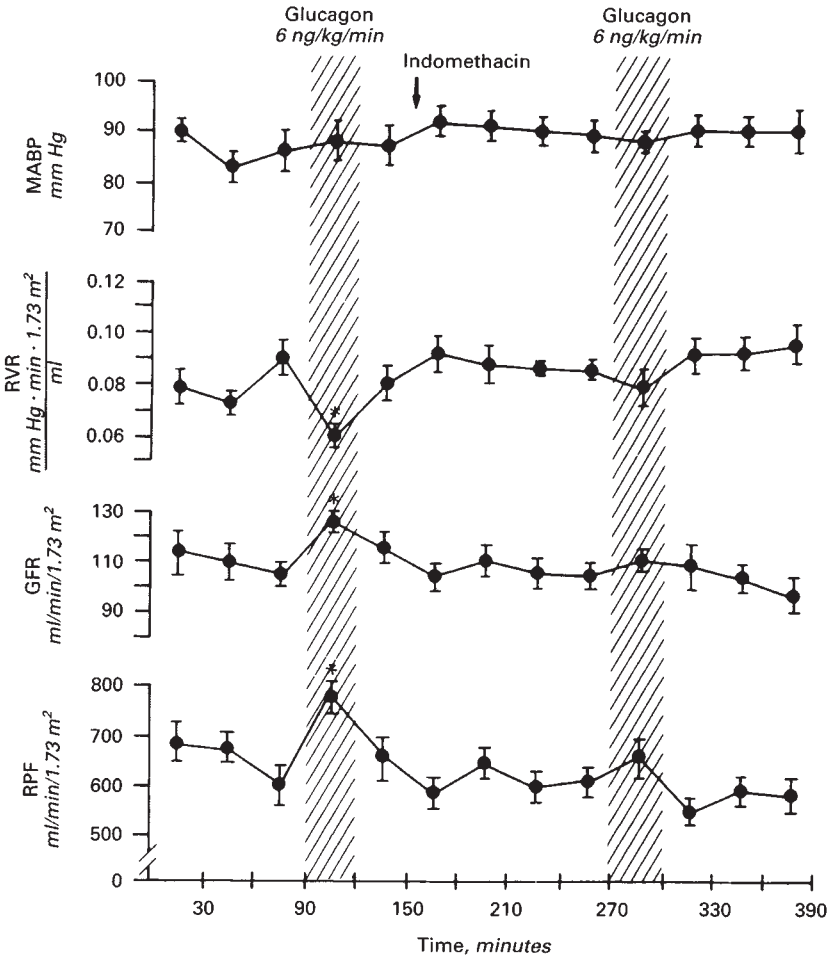

Fig. 3. *Renal hemodynamics and mean arterial blood pressure in the subjects who received glucagon infusions and ingested indomethacin (150 mg) (Study 4, N = 6).* Symbols are defined in the legend to Figure 1. Significantly different from the mean value of the two baseline clearance periods that immediately preceded the infusion: $^{*}P < 0.05$, $^{**}P < 0.01$.

with a different chemical structure, ibuprofen, could also block the arginine induced increase in RPF and GFR and decrease in RVR. During the first arginine infusion in this study, RPF and GFR rose by +36.8 ± 3.3% ($P < 0.01$) and +18.7 ± 2.3% ($P < 0.05$), respectively (Fig. 2, Table 1). After ibuprofen, 800 mg, was ingested, the arginine infusion did not induce a significant rise in RPF (+15.2 ± 3.4%, P:NS) or GFR (+1.9 ± 2.4%, P:NS). RVR decreased by −28.3 ± 5.8% ($P < 0.05$) during the first infusion, but the change during the second infusion, −18.6 ± 8.4%, was not statistically significant. The difference in the fall in RVR between the two arginine infusions was not significant ($P = 0.283$).

In clearance period 5, immediately after the first arginine infusion was terminated, RPF, GFR and RVR returned to baseline values. During clearance period 11, immediately after cessation of the second arginine infusion, RPF and GFR tended to fall to levels below baseline (−15.1% and −12.0%, respectively), and RVR tended to remain above the baseline (+9.5%) (Fig. 2, Table 1); however, none of these changes was statistically significant. During clearance periods 12 and 13, RPF, GFR and RVR returned progressively to baseline values (Fig. 2). Filtration fraction and mean arterial blood pressure did not change significantly during the study.

Study 4 (glucagon-indomethacin-glucagon) was carried out to assess 1) whether raising plasma glucagon concentrations to the levels observed after the arginine infusions would increase RPF and GFR and reduce RVR to the same extent as occurred with the arginine infusion, and 2) whether this effect of glucagon could be blocked with an inhibitor of cyclooxygenase. During the glucagon infusion, there was an increase in RPF and GFR of +21.9 ± 5.2% ($P < 0.01$) and +16.5 ± 3.3% ($P < 0.05$), respectively (Fig. 3, Table 1). RVR decreased by −25.3 ± 5.1% ($P < 0.05$). After pretreatment with indomethacin, the second glucagon infusion did not cause a significant change in RPF, GFR or RVR (Fig. 3, Table 1). Thus, the glucagon induced changes in RPF, GFR and RVR were blocked by the ingestion of indomethacin, 150 mg.

During each of the arginine infusions in Studies 1 to 3, the plasma glucagon levels rose rapidly, regardless of whether the subjects were pretreated with a cyclooxygenase inhibitor (Table 2). In Study 4, the rise and maximum plasma glucagon levels during the two glucagon infusions were virtually identical to the increase that occurred with the arginine infusions. However, plasma glucagon fell rapidly to baseline after termination of the glucagon infusion. In contrast, the decline in plasma glucagon was slower after cessation of the arginine infusion. This suggests that during the rather slow fall to baseline of plasma arginine after the arginine infusion, there may have been continued stimulation of glucagon secretion.

Plasma levels of epinephrine and norepinephrine were not

Table 2. Plasma glucagon (pg/ml) levels before (baseline, BL) during and after the arginine or glucagon infusions

Study	First infusion					Second infusion				
		Infusion[c]		After infusion			Infusion[c]		After infusion	
	BL	15 min	30 min	45 min	60 min	BL	15 min	30 min	45 min	60 min
1 ARG-ARG (Control)	137 ± 19[d]	425 ± 83[b]	584 ± 129[b]	374 ± 101[a]	295 ± 95[a]	148 ± 16	433 ± 10[b]	514 ± 70[b]	360 ± 90[b]	288 ± 92[a]
2 ARG-INDO-ARG	169 ± 20	456 ± 95[b]	662 ± 158[b]	395 ± 84[a]	306 ± 82[a]	254 ± 64	457 ± 90[b]	567 ± 90[b]	381 ± 82[b]	283 ± 66[a]
3 ARG-IBU-ARG	169 ± 17	415 ± 44[b]	632 ± 43[b]	244 ± 28[a]	183 ± 21	152 ± 28	403 ± 57[b]	603 ± 46[b]	230 ± 49[a]	144 ± 17
4 GLUC-INDO-GLUC	183 ± 10	476 ± 41[b]	630 ± 60[b]	174 ± 14	178 ± 14	174 ± 17	454 ± 71[b]	648 ± 86[b]	184 ± 20	167 ± 12

Compared to respective baseline [a] $P < 0.05$, [b] $P < 0.01$.
[c] Infusions were given from 0 to 30 minutes.
[d] Mean ± SEM

Table 3. Plasma norepinephrine and epinephrine concentrations in response to the two arginine infusions in Study 1 and Study 2

Study	Plasma *pg/ml*	First arginine infusion[a]				Second arginine infusion[a]			
		BL	15 min	30 min	45 min	BL	15 min	30 min	45 min
1 ARG-ARG	Norepinephrine	385 ± 99[b]	457 ± 106	518 ± 135	530 ± 143	452 ± 123	433 ± 105	448 ± 111	474 ± 127
	Epinephrine[c]	33 (<20–51)	42 (<20–125)	20 (<20–28)	34 (24–36)	36 (<20–103)	29 (<20–31)	26 (<20–73)	29 (<20–59)
2 ARG-INDO-ARG	Norepinephrine	291 ± 42	308 ± 36	309 ± 43	321 ± 35	259 ± 77	277 ± 71	302 ± 100	266 ± 46
	Epinephrine[c]	20 (<20–73)	20 (<20–24)	22 (<20–27)	20 (<20–31)	22 (<20–58)	42 (<20–73)	32 (<20–83)	30 (<20–76)

[a] Infusions were given from 0 to 30 minutes.
[b] Mean ± SEM.
[c] Results for epinephrine levels are depicted as median and range since some values were below the detection limit of the assay (20 pg/ml).

Table 4. Urine 6-keto-$PGF_{1\alpha}$ excretion before (baseline) and during the two arginine infusions in Study 1 and Study 2

	Study	First baseline	First ARG infusion	Second baseline	Second ARG infusion
1	ARG-ARG (Control)	137.1 ± 27.1	115.1 ± 26.5	109.7 ± 29.6	97.5 ± 21.6
2	ARG-INDO-ARG	260.9 ± 60.2	197.7 ± 38.4[a]	90.8 ± 15.5[b,c]	79.8 ± 15.2

Data are mean ± SEM.
Compared to first baseline [a] $P < 0.05$, [b] $P < 0.01$
Compared to first arginine infusion [c] $P < 0.05$

altered by the arginine or glucagon infusions, nor by indomethacin or ibuprofen (Table 3). Similarly, neither the arginine or glucagon infusions nor the cyclooxygenase inhibitors affected MABP (Figs. 1 to 3).

In Study 1 (control study), the baseline urinary excretion of 6-keto-$PGF_{1\alpha}$ was 137.1 ± 27.1 pg/min (Table 4). The urinary excretion of this metabolite did not change significantly during the first arginine infusion, the subsequent second baseline measurement, or the second arginine infusion. In Study 2, the baseline urinary excretion of 6-keto-$PGF_{1\alpha}$ was greater than in the Study 1 patients. During the first arginine infusion in Study 2, the urinary excretion of this metabolite fell significantly, by 19.1 ± 5.3% ($P < 0.05$). At the second baseline, two hours after pretreatment with indomethacin, urinary excretion of 6-keto-$PGF_{1\alpha}$ had fallen further, by 65.1 ± 5.3% of the initial baseline values ($P < 0.05$) and by 54.4 ± 4.7% ($P < 0.05$) of the values observed during the first arginine infusion (Table 4). During the second arginine infusion, there was a tendency, not significant, for urinary 6-keto-$PGF_{1\alpha}$ excretion to fall further. In all of these studies, the hematocrit did not change significantly during the arginine or glucagon infusions.

Discussion

The rise in RPF and GFR during the arginine infusion was similar to the changes observed in other studies in which individuals received a protein load or an infusion of amino acids [1, 3–6, 13]. However, the mechanisms which mediate the acute renal response to a protein or amino acid load are not well defined. Amino acid infusions are considered a model for protein loading or ingestion of high protein diets with regard to their effects on renal perfusion and filtration. We used the single amino acid arginine, because we have previously characterized some of the systemic and renal effects of short-term arginine infusions [6].

These series of four studies were carried out to further evaluate the mechanisms responsible for the arginine induced acute rise in RPF and GFR. Study 1, in which normal adults received a 30 minute intravenous infusion of arginine HCl, 250 mg/kg, on two occasions separated by a 150 minute interval, was carried out as a control experiment to characterize the normal response to the intravenous arginine load. During both infusions, there was a transient rise in RPF and GFR and fall in RVR; the magnitude of each of these changes was the same in both infusions. These findings suggest that in Studies 2 through 4, any changes in response to the second arginine HCl infusion are not due to time dependent factors but are due to the manipulations carried out between the first and second infusions. Previous studies indicate that the changes in renal function during arginine HCl infusion are not due to the osmotic, chloride or acid load [6].

In a previous study we observed that growth hormone does not play a role in the acute rise in RPF or GFR following an infusion of arginine HCl [6]. During infusion of arginine, patients with growth hormone deficiency displayed an increase in RPF and GFR that was similar to normal individuals. Moreover, in the normal individuals, there was no exact temporal relationship between the increase in plasma growth hormone with the arginine infusion and the rise in RPF and GFR. This is in contrast to the response of plasma glucagon which rose concurrently with the increase in renal hemodynamics in the normal as well as growth hormone-deficient subjects [6].

Premen and coworkers reported evidence that, in dogs, glucagon mediates the protein-induced acute rise in RPF and GFR [15, 29]. Somatostatin blocked the increase in RPF and GFR following a protein load; this suggests that peptide hormones are mediating factors. When glucagon was infused into a peripheral vein, RPF and GFR increased only when plasma glucagon levels rose above the values that are obtained with a protein load. However, when a small dose of glucagon was infused into the portal vein of these dogs, RPF and GFR rose. These studies suggest that plasma glucagon, in postprandial concentrations, does not act directly on the kidney to increase renal function but may act through the liver, possibly by stimulating the release of another humoral factor. This other factor has been called glomerulopressin [29–31].

Castellino, Coda and DeFronzo demonstrated that in normal adults who were infused with a mixture of amino acids, the rise in RPF and GFR could be blocked by somatostatin infusion [13]. The subjects then received, in addition to the amino acid and somatostatin infusions, a combined infusion of glucagon, growth hormone and insulin that raised plasma levels to the same values observed with the amino acid infusion alone. Under these circumstances, RPF and GFR rose to the same magnitude as with the infusion of amino acids alone without somatostatin. A similar observation was reported by Palmore who infused alanine, with or without somatostatin, into dogs [14]. Somatostatin significantly lowered the alanine induced rise in RPF and GFR. A glucagon infusion of 7 to 11 ng/kg/min increased RPF and GFR to the same extent as with the alanine infusion. However, the author did not report the plasma glucagon concentrations that were attained with the alanine or glucagon infusions. Parving and coworkers demonstrated a significant rise in RPF and GFR without a change in the filtration fraction in subjects with insulin-dependent diabetes mellitus who received glucagon infusions of 10 or 4 ng/kg/min [32].

Amiel and coworkers infused a mixed amino acid solution into pancreatectomized patients. In contrast to normal subjects, RPF and GFR did not increase in these patients [33].

The foregoing studies provide evidence that glucagon is a mediator of the protein or amino acid induced acute rise in RPF and GFR. The present study extends these observations by demonstrating that 1) acute intravenous infusions of small quantities of glucagon do raise RPF and GFR in humans, and 2) this effect occurs at plasma glucagon concentrations that may occur in response to an amino acid load (Study 4, Table 2). During the glucagon infusion, there was a rise in RPF and GFR and a fall in RVR which were of similar magnitude to that observed with the arginine infusion (Table 1). These findings provide strong evidence that physiological concentrations of glucagon increase RPF and GFR and lower RVR in humans. The data do not indicate whether the effect is due to a direct action of glucagon on the kidney.

In order to assess whether the effect of the amino acid infusion on RPF and GFR requires the presence of prostaglandins, we carried out Studies 2 and 3 in which the subjects received cyclooxygenase inhibitors. Indomethacin, an indole derivative, and ibuprofen, which is derived from propionic acid, were selected because they have different chemical structures. Hence, it is more likely that any effect on renal function would be due to their common action on cyclooxygenase. With each drug, there was a marked reduction in the arginine induced rise in RPF and GFR and in the fall in RVR. These findings do not indicate whether prostaglandins exert a permissive effect on the changes in renal function or whether there is an elevation in prostaglandin levels which directly increases RPF and GFR. This study also does not rule out the possibility that cyclooxygenase inhibitors block the arginine or glucagon induced effects on renal function indirectly, by suppressing extrarenal synthesis of eicosanoid compounds.

It is puzzling that although cyclooxygenase inhibitors suppressed the arginine induced rise in RPF and GFR, the arginine infusions in the control study and in the studies where it was given before the administration of the cyclooxygenase inhibitors did not increase the urinary excretion of 6-keto-$PGF_{1\alpha}$ (Table 4). 6-Keto-$PGF_{1\alpha}$ is a stable metabolite of prostacyclin. Hence, these findings may indicate that prostacyclin does not play a role in the arginine induced change in renal hemodynamics.

It is possible that other eicosanoids are responsible for mediating the changes in RPF, GFR and RVR. Alternatively, the urinary excretion of 6-keto-$PGF_{1\alpha}$ may not necessarily reflect the concurrent activity of prostacyclin at the site in the glomerulus where this compound may lower RVR and increase RPF and GFR. It is noteworthy in this regard that in human kidney, prostaglandin-endoperoxide synthetase and cyclooxygenase have been localized mainly in the tubular, peritubular and interstitial areas [34]. Thus, if normally there is much greater synthesis of prostaglandins in the renal tubules, an increase in the rate of synthesis in the glomeruli may not lead to significant changes in the urinary excretion of prostaglandin metabolites. Moreover, the lack of rise in urinary 6-keto-$PGF_{1\alpha}$ also does not rule out the possibility that prostacyclin exerts a permissive effect on the arginine induced increase in renal

hemodynamics. In this regard, in Study 2 after the individuals ingested indomethacin, there was a significant reduction in the urinary excretion of the PGI_2 metabolite before the second arginine infusion (Table 4). Although the urinary excretion of 6-keto-$PGF_{1\alpha}$ was falling before the indomethacin was ingested, the magnitude of the decrease was greater after the indomethacin treatment.

It would have been helpful if data on the urinary excretion of PGE_2 were available in this study. However, for technical reasons we were unable to measure PGE_2 in the urines of our subjects. It is pertinent that Ruilope and associates infused mixed amino acids into humans at a slower rate than was used in our study and demonstrated a significant rise in urinary PGE_2 excretion [35].

Hostetter reported no increase in urinary PGE_2 excretion in normal subjects who ingested a protein load, even though their RPF and GFR rose [4]. He concluded that prostaglandins were not likely to be mediators of the vasodilation during protein loading. However, inhibitors of prostaglandin synthesis were not used in these studies. Levine and coworkers [36] demonstrated a significant increase in both GFR and the rate of glomerular synthesis of PGE_2 and 6-keto-$PGF_{1\alpha}$ in rats in response to an oral protein load.

The finding that indomethacin also blocked the glucagon induced rise in RPF and GFR and the decrease in RVR indicates that prostaglandins are necessary for these renal hemodynamic effects of glucagon. This is consistent with the hypothesis that an amino acid load stimulates the release of glucagon which, in turn, increases RPF and GFR, and that this latter effect requires the active or permissive action of prostaglandin compounds.

TerWee and coworkers infused dopamine intravenously into normal individuals and observed an increase in RPF and GFR and a decrease in filtration fraction [7]. As in the present study, these authors did not find a change in filtration fraction when amino acids were infused. They concluded that catecholamines increase RPF and GFR by a different mechanism than the amino acids. In the present study we observed no increase in plasma norepinephrine and epinephrine concentrations during the amino acid infusions, and indomethacin had no effect on these plasma levels. These findings support the observations of TerWee and coworkers that systemic catecholamines do not play a role in the amino acid-induced effects on renal hemodynamics. However, our findings do not exclude the possibility that renal nerves may exert a mediating effect.

In summary, the present studies provide evidence that prostaglandins are modulators of the arginine induced rise in RPF and GFR. In normal subjects a low-dose infusion of glucagon which raises plasma glucagon to the same levels as with the arginine infusion significantly increases renal hemodynamics. The glucagon induced rise in RPF and GFR can be blocked by indomethacin. Thus, these studies indicate that glucagon and prostaglandins are mediators or modulators of the amino acid-induced changes in renal hemodynamics.

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